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CONTENTS

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The Experiment Station
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FACTORS associated with breeding efficiency in cattle have received a great deal of attention and study since men first became interested in improvement of domesticated animals. Increased breeding efficiency aims at the ideal condition of every cow in the herd having a calf each year. This leads to more economical production and, in the long run, to greater total production. Now the development and use of artificial insemination have stimulated the study of specific factors that influence efficiency in dairy-cattle breeding, in the hope that through a better understanding of certain phases of reproduction, modern dairy farming may be able to avoid the serious losses which often result from delayed conception. Despite a vast amount of research on breeding efficiency, many questions pertaining to rates of conception have not yet been answered. It was the purpose of this experiment to provide answers to some of these questions.

In recent years, there has been a greater appreciation of the loss resulting from poor breeding efficiency in the bovine. Both quantity and economy of production have been given more emphasis. After a rigid health program has been followed for a long time, and diseases such as contagious abortion, trichomoniasis, and various forms of vaginitis have been eliminated from the herd, it is a surprise to many farmers, livestock breeders, and research men that the breeding efficiency is lower and the average number of services required per conception is higher than was anticipated. Some farmers and breeders, probably because they are poor observers or do not keep accurate records, believe that all their females conceive at the first service. Those dairy-cattle breeders who keep accurate records and closely observe their animals realize that, with the present knowledge of breeding, a herd in which good results are obtained will require an average of 1.4 to 2.0 services per conception. If this is stated on the basis of the percentage of cows that conceive with the first service or insemination, the range is from sixty to seventy.

Review of Literature

MANY references are available bearing on the breeding efficiency of dairy cattle. The research so far reported serves as a basis for a normal standard which is useful in judging the breeding efficiency in herds under practical management, and which is helpful in properly evaluating the results from breeding experiments.

To facilitate presentation and reading, the references in which the various authors reported data on breeding efficiency are presented in tabular form and are grouped for natural service in Table I and for artificial insemination in Table II.

The tables include only the fertile females, but a limited number of sterile cows and heifers can be regarded as a normal occurrence so there is a certain amount of error in the figures as regards an average herd. Fox (16) reported that five per cent of the females have to be sold as

Table I.—Breeding Results from Natural Services

Reference	Fertile Cows	Services per Conception to Fertile Cows	Cows Conceiving at Various Services				
			1st	2nd	3rd	4th	5 or more
	No.	No.	Per cent	Per cent	Per cent	Per cent	Per cent
Gowen (18)	1801	1.32	78.4	15.4	4.5	1.0	0.7
Eckles (14)	1151	2.52	47.4	22.4	12.7	6.8	10.7
Gowen and Dove (19)	7679	1.63	65.3	19.5	8.5	3.1	3.6
Miller and Graves (30)	730	2.88	41.1	18.8	12.5	8.0	19.6
Knoop and Hayden (27)	379	1.87	58.8	21.1	10.3	4.2	5.6
" " "	340	1.53	65.6	24.1	6.1	1.7	2.5
Dawson (13)	1179	2.49	.....	.....	.....	.....	.....
Morgan and Davis (32)	1375	2.21	.....	.....	.....	.....	.....
Herman (22)	150	1.66	.....	.....	.....	.....	.....
Herman and Ragsdale (23)	1197	1.53	.....	.....	.....	.....	.....
Backstrom (2)	79	1.91	.....	.....	.....	.....	.....
Bowling, Putnam, Ross (5)	1822	2.02	.....	.....	.....	.....	.....
Erb, Wilbur, Hilton (15)	922	1.42	72.1	18.7	6.3	2.3	0.6
Ward (40)	2619	1.58	62.7	23.7	9.0	3.7	0.9
Seath and Staples (35)	934	1.70	65.7	18.2	8.4	4.1	3.6
Hofstad (24)	309	1.65	.....	.....	.....	.....	.....
Totals and Averages	22684	1.79					
	16555	.....	64.3	19.9	8.5	3.4	3.9

sterile, Eckles (14) stated that 6.3 per cent of the individuals of all ages in a dairy herd were sterile, and added that 7.4 per cent of the heifers did not conceive. Ward (40) found 204 sterile cows for 2,619 pregnant individuals; that is, 7.2 per cent of the cows failed to conceive. Miller and Graves (30) reported that 5.8 per cent of females were non-breeders.

The rate of sperm travel and survival of the spermatozoa in the genital tract of the cow may influence the results obtained from breeding. Andreev (1) stated that the spermatozoa survived in the reproductive tract for a relatively short time which he fixed at approximately 24 hours, and he believed that this short survival period may be partially responsible for some conception experiences. Kirillov (25) found that the majority of the spermatozoa were dead 22 to 25 hours after mating. Beschlebnov (4) observed 22 cows and found that motility remained satisfactory for 24 to 30 hours after service but that only a few motile spermatozoa were observed after 40 hours. Brewster, May and Cole (7) studied 14 females and concluded that the minimum time required for sperm to reach the Fallopian tubes was four hours and fifteen minutes in heifers, and about five hours and three minutes in mature cows, the difference in time being due to the difference in length of the genital tract. These authors observed no significant differences in the rate of sperm travel with cows inseminated in estrus as compared to cows out of estrus. Sergin, Kuznecov, Kozlova and Nesmejanova (36) slaughtered 35 cows after service and reached the conclusion that the spermatozoa survived several days in the cervix of the cow.

Time of ovulation and the optimum time to inseminate have received attention by various researchers, but little is available from carefully controlled experiments as to the best time to inseminate. Gowen (18) reported as early as 1918 that the best time for successful service was 10 to 24 hours after onset of estrus, although he reported one successful service 41 hours after discovery of heat. Zupp (43) reported that ovulation in the cow took place 30 to 65 hours after the onset of estrus, while Hammond (20) stated that ovulation occurred somewhere between 24 and 48 hours after the beginning of estrus. Cole (8) stated that ovulation usually occurred one day post-estrus. Miller and associates (31) recovered a fertilized egg from the uterus of a cow 48 hours after the appearance of estrus and they believed that ovulation and fertilization may be completed within 48 hours after the start of estrus in the cow. Kufarev (28) believed the optimum moment of insemination was during the second half of estrus when the vagina contained large quantities of mucus.

Andreev (1) found it advantageous to inseminate cows twice during a heat period because ovulation occurred 30 to 40 hours after the onset of estrus, and spermatozoa did not live long enough in the genital tract of the female to fertilize the egg if the semen was introduced into the cow early in the heat period. Kirillov (25) divided a herd into two parts, and 60 per cent of the cows mated at the beginning of estrus had to be rebred, but only 26 per cent of the cows mated 18 to 24 hours after beginning of estrus required subsequent service. Beschlebnov (4) advised against insemination of cows during the first 10 hours of heat. Bartlett and Perry (3) stated that inseminations during full and late estrus showed the highest rate of conception. Full estrus was defined as eight to 12 hours after, and late estrus as 12 to 24 hours after the onset of heat. Henderson (21) reported that in the New Jersey artificial breed-

Table II.—Breeding Results from Artificial Services

Reference	Fertile Cows	Services per Conception to Fertile Cows	Cows Conceiving at Various Services				
			1st	2nd	3rd	4th	5 or more
	No.	No.	Per cent	Per cent	Per cent	Per cent	Per cent
Bartlett and Perry (3)	1950	1.67	54.8	.....	.....	.....	.....
Davis and Williams (12)	73	1.30	.....	.....	.....	.....	.....
Henderson (21)	568	1.91	.....	.....	.....	.....	.....
Herman (22)	503	1.80*	.....	.....	.....	.....	.....
"	150	1.59	.....	.....	.....	.....	.....
Lasley, Montgomery, McKenzie (29)	210	1.54	.....	.....	.....	.....	.....
Backstrom (2)	79	1.62	.....	.....	.....	.....	.....
Davis, Trimberger, Underbjerg (9)	107	1.69†	61.7	20.5	7.5	8.4	1.9
" " "	122	1.54	67.2	20.5	8.2	1.6	2.5
Davis, Underbjerg, Trimberger (10)	103	1.45	68.9	20.4	7.8	1.9	1.0
" " "	67	1.63‡	64.2	17.9	10.4	6.0	1.5
Davis, Underbjerg, Williams (11)	112	1.38	.....	.....	.....	.....	.....
Kissileff (26)	159	1.77	.....	.....	.....	.....	.....
Underbjerg and Davis (37)	260	1.50	.....	.....	.....	.....	.....
Willett, Fuller, Salisbury (42)	318	1.53	.....	.....	.....	.....	.....
" " "	758	1.40	.....	.....	.....	.....	.....
Underbjerg, Davis, Spangler (38)	365	.....	63.5	.....	.....	.....	.....
Totals and Averages	5539	1.63					
	2714	.....	57.5				
	399	.....	65.7	20.0	8.3	4.2	1.8

* Included some infertile cows.
† Samples obtained by massage method.
‡ Vaginal inseminations with capsule gun.

ing ring the stage of heat of the cow was roughly recorded at the time of 717 inseminations. The results were as follows:

Stage of Heat	Number of Inseminations	Number Pregnant	Number not Pregnant	Services per Conception
Early	59	25	34	2.36
Full	366	203	163	1.80
Late	227	121	106	1.88
Post	65	30	35	2.17
	717	379	338	1.89

Henderson admits that the above was a very rough classification, and probably a good proportion of the classification would not be accurate because the farmers merely estimated the stage of heat. Herman (22), using a similar classification, reported as follows:

Time of Inseminations Hours after first signs of heat	Number of Inseminations	Number of Cows Settled	Average Inseminations per Conception
4-12 hours	920	536	1.71
12-24 hours	219	100	2.19
24-48 hours	192	90	2.13
48-60 hours	27	10	2.70

He reached the conclusion that fewer services per conception were required when cows were bred while showing evidences of "active heat" and that a smaller number of inseminations per conception were required when breeding occurred within 12 hours after the first signs of heat.

Research on artificial insemination in the United States Department of Agriculture (39) indicated that artificial insemination can be used successfully for a short period after estrus has passed.

Gerasimova (17) observed ovulation in 125 cows and found it occurred during the interval of 16 to 38 hours after the onset of estrus with an average of 27 hours and 50 minutes. The most frequent interval, 89.1 per cent of the cases, was 20 to 32 hours after the onset of estrus. Werner, Casida and Rupel (41) examined 35 heifers and determined that in 30 cases ovulation had occurred not later than the third half-day following the beginning of heat. They recommended insemination late in the heat period or soon after the close of heat as the optimum time for service.

Nalbandov and Casida (33), from examinations of 15 estrual periods, found a variation of time of ovulation from 2½ to 22¾ hours, with an average time interval of 11¾ hours from end of heat to ovulation. Brewster, May and Cole (7) and Brewster and Cole (6) made 83 rectal examinations to determine ovulation on 47 animals. Three cows did not ovulate, and 53 of the 70 ovulations were included in computing averages because the remaining 17 were not examined within the two-hour time limitation. The average time of ovulation from the end of estrus was 13.57 ± 0.68 hours. A significant difference of 3.04 hours was found between heifers and cows. In heifers, 16 ovulations averaged 11.4 ± 0.99 hours as compared to 37 ovulations in cows with an average of 14.48 ± 0.84 hours. Eighty-nine per cent of the ovulations occurred between six and nineteen hours after the end of estrus. Time of day or kind of breed had no influence on the time of ovulation.

Nalbandov and Casida (34) determined the time of ovulation for 70 heat periods in 22 cows and found that in 77 per cent of the cases, ovulation occurred 10 to 18 hours after the end of heat. In nine per cent of the cases it occurred earlier than 10 hours after estrus, and in 14 per cent of the cases it occurred later than 18 hours after estrus. The authors stated that for the latter group the chances were relatively poor for the spermatozoa to survive in the genital tract of the cow from mating some time during the heat period until the egg was available for fertilization, and this would result in reduced fertility. The authors stated that cows should be bred near the end of the heat period, as this increased the chances of the spermatozoa living until the egg was available for fertilization.

Experimental Methods and Procedure

THIS study is based on the breeding record obtained from Jersey, Guernsey, Ayrshire, and Holstein females in the University of Nebraska dairy herd. The experimental groups included both heifers and cows. Although it was pointed out in the review of literature, and also indicated by the breeding records at the University of Nebraska, that heifers require more services per conception than do cows, it was considered best to include both because it would be more comparable to conditions on the average farm where both cows and heifers make up the breeding herd. Only those females were selected that as far as could be determined had sound reproductive organs and were free from any disturbances or abnormalities in their genital tract, as indicated by examination per rectum. All animals in the entire herd were negative to Bangs disease, based on regular tests three times yearly. The first cow on this breeding experiment was bred December 1, 1937, and the last one on October 29, 1940. All females in which conception took place either dropped a normal calf or aborted.

The early detection of heat at the start of estrus in the females to be bred experimentally for this study was very important, hence a heat expectancy list was used. It consisted of a card on which were the names of all open females and those bred but not pregnant, followed by the dates on which they were expected to be in heat. The individuals were observed very closely a few days before and after the twenty-first day of the preceding estrus or service date. During the time of this study the senior author was the herdsman in charge of the University of Nebraska dairy herd, which made it possible for him to observe each animal carefully and frequently. Every animal in the herd was inspected at least three times each day—morning, noon, and evening—while individuals due in heat were observed every two hours. Consequently, the start of estrus was usually known within an interval of one hour.

A cow or heifer was considered to be in heat if she was complacent when mounted by other females in the herd. Accordingly, throughout the remainder of this report, the term estrus is used to indicate the fact that the cow or heifer was complacent when mounted by other individuals, and "mounting" and "standing" were always taken as indications of heat. The majority of the bovine females remained in heat about 18 hours. This figure was used for dividing the individuals into groups bred at the start, at the middle, and toward the end of the estrus periods. All services to cows after estrus had ceased were based on hours of service after end of estrus.

This procedure was followed because it was possible to determine the end of estrus more accurately and in considerably less time than was possible for the start of estrus. Furthermore, from a practical standpoint, the end of estrus as a basis for stating the expected possible percentage of conceptions would be more useful to a farmer, because many times he does not know when a cow begins to be in heat, but it is relatively easy for him to determine when heat ceases. To determine the end of estrus, the females were tested every two hours, with three final tests made every two hours after the end of estrus. Thus the individual was tested the second, the fourth, and the sixth hour after estrus in order to guard against the possibility of some cows going out of heat for a short time and later coming back into estrus again. A test for estrus every two hours made it possible to determine within an hour the time of cessation of estrus, and if a female was in estrus at one test and out two hours later, the mid-point between these two tests was taken as the end of estrus. In border-line cases when a female would stand only half of the time she was mounted and move about at other times, the test was repeated after one hour.

The different stages of estrus during which the females were allowed services were as follows:

1. **Start of Estrus.** Individuals in this group were inseminated the first six hours of estrus and were required to be in heat 12 hours after the insemination.
2. **Middle of Estrus.** Individuals were required to be in heat at least six hours before insemination and had to be in heat six hours after insemination.
3. **End of Estrus.** Females in this group were required to be in heat at least 12 hours before insemination and were required to be out of heat six hours after insemination.
4. **Bred and Rebred in 24 Hours.** These females were bred in full heat, as in the second group, but they were given another insemination after 24 hours.
5. **Six Hours After Estrus.** Individuals in this group were inseminated six hours after estrus ended.
6. **Twelve Hours After Estrus.** Inseminations were made 12 hours after estrus ended.
7. **Eighteen Hours After Estrus.** Females were bred 18 hours after estrus ended.
8. **Twenty-four Hours After Estrus.** Individuals here were inseminated 24 hours after estrus ended.
9. **Thirty-six Hours After Estrus.** These females were inseminated 36 hours after cessation of heat.
10. **Forty-eight Hours After Estrus.** Inseminations were given 48 hours after estrus had ceased.

Another large group, although not an experimental group, was available for comparison. It consisted of the females bred during heat as routine procedure in the university herd without any set time as to stage of estrus.

All services were by means of artificial inseminations with fresh semen of good quality from disease-free bulls with good breeding records, and one cc. of semen was delivered into the cervix. All inseminations were cervical because this experiment was started before the technique of passing the inseminating tube through the cervix for uterine insemination was commonly practiced.

Discussion

WHEN the individuality of each cow was well known it was not difficult to detect cows in heat by observing certain changes in their behavior. At the onset of estrus most of the animals showed a great deal of restlessness and frequently were standing in the stalls while the others were lying down, or in the open lot they would feed a short time and wander about intermittently. Also a certain sparkle or alertness was noticeable in the eyes of the individuals in heat. A definite spring, so to speak, was in their step and they had greater muscular coordination as they moved about in the lot. Cows in heat occasionally twitched their tails, and bawled frequently. Usually a mucus discharge was seen coming from the vulva, and often a marked out-pouring of clear, viscid, cohesive mucus was observed.

The females tested for heat every two hours were observed to go out of heat gradually with a sort of in-between stage when they were half in and half out, and for this reason it was possible to determine the end of estrus only within an hour. The mid-point between the two-hour tests would be very close to the actual time that estrus ended. If the individual stood only part of the time when mounted, the test was repeated within an hour because the indifferent attitude was an indication that the individual was passing over the heat period. The females in heat were complacent at all times when mounted by others, and would move about and stand alternately only when they were coming in or going out of heat. After some experience it was possible in nearly every case to determine when a female had passed out of heat by observing closely the individual: she was very much depressed, lacked alertness, and moved about slowly as compared to the energetic movements during estrus. However, in every case, the individuals were checked by the "mounting" and "standing" method.

Results

A TOTAL of 295 cows and heifers were bred in the experimental groups during the various stages of estrus. Each of the groups contained 25 females except the groups bred during the middle of estrus, toward the end of estrus, and six hours after estrus ended: these had 40 individuals in each group. The individuals were entered in the groups at the time of breeding and none were taken out later. As already described, the genital organs of each female were carefully examined before she was placed in an experimental group and those with cystic or unsound ovaries, pyometra or vaginitis, were not included in the experimental groups, nor were the cows that had a history of a marked case of metritis at the time of previous calving. There were no cows eliminated from any of the groups after they had been bred.

The degrees of sexual excitement exhibited by the females in heat can be characterized as intense, moderate, feeble, and silent. All shades in between these degrees were observed, and it was considered an impossibility to divide them on a percentage basis. Twenty-two per cent of the females in which conception failed to take place did not return in heat at the next expected heat period. The majority of these cases apparently were due to a very feeble or silent heat period rather than lack of detection, because these individuals were closely watched and observed very frequently. Some of these females were examined per rectum for several days during and a few weeks after the expected estrus.

The development of follicles on the ovaries during the time of expected estrus, and this followed by the development of a new *corpus luteum* when examined two weeks later, indicated ovarian function but a silent heat period. No intermittent heat periods in which the animals were in heat, out of heat, and then came back in again were found in the tests made among these groups.

The breeding results in percentages of conceptions obtained in the females bred at various stages of estrus are presented in Table III. During the period of this experiment (one month less than three years) the conceptions were tabulated for all the cows in the university herd bred artificially, and it was found that from a total of 194 cows bred, 123 conceived, which resulted in a conception percentage of 63.40. Services to females at the start of estrus, and those 12 hours after estrus ended and later than this, produced poor breeding results and the differences were found significant when statistical methods were applied.

Table III.—Breeding Results in Females Bred Experimentally at Various Stages of Estrus and in Females Bred Artificially in the University Herd.

Time of Service	Cows Bred No.	Cows Conceiving from One Service	
		No.	Per cent
Start of estrus	25	11	44.00
Middle of estrus	40	33	82.50
Bred at middle of estrus and rebred in 24 hours	25	21	84.00
End of estrus	40	30	75.00
Artificial routine breeding	194	123	63.40
6 hours after estrus ended	40	25	62.50
12 hours after estrus ended	25	8	32.00
18 hours after estrus ended	25	7	28.00
24 hours after estrus ended	25	3	12.00
36 hours after estrus ended	25	2	8.00
48 hours after estrus ended	25	0	0.00

Consideration of rates of conception in cows bred at various stages of estrus may prompt the question of whether there is any influence on the sex of the offspring. The number of abortions, number of calves, sets of twins, and the sex of the offspring in numbers and percentages are summarized for the various groups in Table IV. There were no significant deviations from the expected numbers for each sex when statistical methods were applied, except in the group bred six hours after estrus, and little importance can be attached to this since the numbers involved are comparatively small.

Summary and Conclusions

A BREEDING experiment with 295 dairy cows and heifers of the Jersey, Guernsey, Ayrshire, and Holstein breeds was conducted in the University of Nebraska dairy herd. The females were given artificial services at various stages of estrus to determine the effect of time of service upon conception. The breeding results, expressed as percentages of conception from one insemination in the females bred at various stages of estrus, were as follows: start of estrus, 44.0; middle of estrus, 82.5; middle of estrus and rebred in 24 hours, 84.0; end of estrus, 75.0; six hours after estrus ended, 62.5; 12 hours after estrus ended, 32.0; 18 hours after estrus ended, 28.0; 24 hours after estrus ended, 12.0; 36 hours after estrus ended, 8.0; and 48 hours after estrus ended, none conceived. During the 35 months of this experiment, 194 females were bred by artificial

Table IV.—Sex of Offspring for Calves Obtained from Groups Bred at Various Stages of Estrus

Time of Service	Abortions No.	Calves No.	Sets of Twins No.	Males No.	Females No.	Males Per cent	Females Per cent
Start of estrus	0	13	2	6	7	46.15	53.85
Middle of estrus	2	34	3	19	15	55.88	44.12
Bred at middle of estrus and rebred in 24 hours	0	24	3	11	13	45.83	54.17
End of estrus	0	32	2	13	19	40.63	59.37
Artificial routine breeding	3	125	5	66	59	52.80	47.20
6 hours after estrus ended	4	23	2	7	16	30.43	69.57
12 hours after estrus ended	1	8	1	5	3	62.50	37.50
18 hours after estrus ended	2	5	0	3	2	60.00	40.00
24 hours after estrus ended	0	3	0	1	2	33.33	66.67
36 hours after estrus ended	0	2	0	1	1	50.00	50.00
48 hours after estrus ended	0	0	0	0	0	00.00	00.00
TOTAL	12	269	18	132	137	49.07	50.93

insemination in the routine breeding in the university herd, and 123 or 63.4 per cent of the females conceived at the first insemination. This large group can be used as a normal standard for a basis of comparison.

Although the individuals were closely watched and observed very frequently, 22 per cent of the females in which conception failed to take place did not return in heat at the next expected period. Silent heat periods were frequently observed among these females but no intermittent heat periods were found.

The results obtained indicate the potential benefits in higher percentage of conception in females bred during the middle of estrus, toward the end of estrus, and those bred in full heat and rebred in 24 hours. The latter is usually not very practical, especially in artificial breeding rings, but there are occasions when it may be desirable to follow this procedure. The results also show that a good rate of conception is obtained in females bred as late as six hours after the end of estrus. The low rates of conception in experimental groups bred long after the end of estrus indicate that it is seldom practical to breed females later than six hours after estrus ends. Statistical treatment of the data verified these conclusions.

Bibliography

1. *Andreev, E. L.* Insemination of Cows Twice during One Heat Period. Probl. Zivotn. 5:134–138. In: An. Breed. Abs. 5:277. 1937.
2. *Backstrom, K.* Den Konstgjorde Sadesoverforingens betydelse. Kunglige Lantbruksakademiens Tidskrift 79:163–177. 1940.
3. *Bartlett, J. W. and E. J. Perry.* Lessons Learned from Eighteen Months' Experience with Cooperative Artificial Breeding of Dairy Cattle in New Jersey. Amer. Sci. Anim. Prod. Proc. 32:243–245. 1939.
4. *Beschlebnov, A. V.* The Optimal Time of Inseminating Cows during Oestrous. Probl. Zivotn. 2:73–88. In: An. Breed. Abs. 6:291. 1938.
5. *Bowling, G. A., D. N. Putnam and R. H. Ross.* Age as a Factor Influencing Breeding Efficiency in a Dairy Herd. Jour. Dairy Sci. 23:1171–1176. 1940.
6. *Brewster, J. E. and C. L. Cole.* The Time of Ovulation in Cattle. Jour. Dairy Sci. 24:111–115. 1941.
7. *Brewster, J. E., Ralph May and C. L. Cole.* The Time of Ovulation and Rate of Spermatozoa Travel in Cattle. Amer. Soc. Anim. Prod. Proc. 33:304–310. 1940.
8. *Cole, H. H.* A Study of the Mucosa of the Genital Tract with Special Reference to the Cyclic Changes. Amer. Jour. Anat. 46:261–301. 1930.
9. *Davis, H. P., G. W. Trimberger and Gravers K. L. Underbjerg.* Fecundity and Certain Other Characteristics of Fresh and Stored Bovine Semen. Jour. Dairy Sci. 23:532–533. 1940.
10. *Davis, H. P., G. K. L. Underbjerg and George W. Trimberger.* An Improved Method for Artificial Insemination of the Bovine by Vaginal Deposition of the Semen. Amer. Soc. Anim. Prod. Proc. 33:221–223. 1940.
11. *Davis, H. P., G. K. L. Underbjerg and N. K. Williams.* The Effect of Storage Temperature Upon Certain Characteristics of Bovine Semen. Jour. Dairy Sci. 23:1057–1068. 1940.
12. *Davis, H. P. and N. K. Williams.* Evaluating Bovine Semen. I. Influence of the Number of Ejaculations Upon Various Physical and Chemical Characteristics and the Relationship Between Those Factors. Amer. Soc. Anim. Prod. Proc. 32:232–242. 1939.
13. *Dawson, J. R.* The Breeding Efficiency of Proved (Aged) Sires. Jour. Dairy Sci. 21:725–737. 1938.
14. *Eckles, C. H.* A Study of Breeding Records of Dairy Herds. Minn. Agr. Exp. Sta. Bul. 258. 1929.
15. *Erb, R. E., J. W. Wilbur and J. H. Hilton.* Some Factors Affecting Breeding Efficiency in Dairy Cattle. Jour. Dairy Sci. 23:549. 1940.
16. *Fox, Henry P.* Sterility and Impaired Fecundity in Farm Animals. Vet. Jour. 97:41–61. 1941.
17. *Gerasimova, A. A.* Duration of Heat and Time of Ovulation in the Cow. Probl. Zivotn. 12:126–129. In: An. Breed. Abs. 8:32. 1938.
18. *Gowen, John W.* Report of Progress on Animal Husbandry Investigations in 1917. Maine Agr. Exp. Sta. Bul. 274:221–228. 1918.

19. *Gowen, J. W. and W. F. Dove.* Fertility in Dairy Cattle. Maine Agr. Exp. Sta. Bul. 360:170–172. 1931.
20. *Hammond, J.* The Physiology of Reproduction in the Cow. Pp. 9–49. University Press. Cambridge, England. 1927.
21. *Henderson, J. A.* New Jersey Artificial Breeding Results. Jersey Bulletin 58:201. 1939.
22. *Herman, H. A.* Some Factors Affecting the Efficiency of Artificial Insemination of Dairy Cows. Amer. Soc. Anim. Prod. Proc. 32:245–250. 1939.
23. *Herman, H. A. and A. C. Ragsdale.* Artificial Insemination in Dairy Cows. Mo. Agr. Exp. Sta. Res. Bul. 407. 1939.
24. *Hofstad, M. S.* A Study of Breeding Records of One Large Herd of Dairy Cattle. Cornell Vet. 31:379–381. 1941.
25. *Kirillov, V. S.* New Principles of the Organization and Technique of Mating of Cows. Probl. Zivotn. 3:34–42. In: An. Breed. Abs. 5:278. 1937.
26. *Kissileff, A.* Artificial Insemination of Dairy Cattle in Private Practice. Jour. Amer. Vet. Med. Assoc. 96:309–315. 1940.
27. *Knoop, C. C. and C. C. Hayden.* A Study of the Length of Gestation and Service Record of Dairy Cattle. Ohio Agr. Exp. Sta. Bimonthly Bul. 166:8–14. 1934.
28. *Kufarev, A. P.* Insemination of Cows with Undiluted Sperm. Probl. Zivotn. 6:107–108. In: An. Breed. Abs. 4:35. 1935.
29. *Lasley, J. F., J. T. Montgomery and F. F. McKenzie.* Artificial Insemination in Range Cattle. A Preliminary Report. Amer. Soc. Anim. Prod. Proc. 33:102–105. 1940.
30. *Miller, F. W. and R. R. Graves.* Reproduction and Health Records of the Beltsville Herd of the Bureau of Dairy Industry. U. S. Dept. Agr. Tech. Bul. 321. 1932.
31. *Miller, F. W., W. W. Sweet, C. G. Hartmann and W. H. Lewis.* A Study of Ova from the Fallopian Tubes of Dairy Cows with a Genital History of the Cows. Jour. Agr. Res. 43:627–636. 1931.
32. *Morgan, R. F. and H. P. Davis.* Influence of Age of Dairy Cattle and Season of the Year on the Sex Ratio of Calves and Services Required for Conception. Nebr. Agr. Exp. Sta. Res. Bul. 104. 1938.
33. *Nalbandov, A. and L. E. Casida.* The Best Time for Insemination of Dairy Cows. Wis. Agr. Exp. Sta. Bul. 446:15. 1939.
34. *Nalbandov, A. and L. E. Casida.* Ovulation and Its Relation to Estrus in Cows. Jour. Anim. Sci. 1:189–198. 1942.
35. *Seath, D. M. and C. H. Staples.* Some Factors Influencing the Reproductive Efficiency of Louisiana Herds. Jour. Dairy Sci. 24:510. 1941.
36. *Sergin, N. P., M. P. Kuznecov, V. M. Kozlova and T. N. Nesmejanova.* Physico-Chemical Condition in the Genital Tract of the Cow and Survival of Spermatozoa. Dokl. Akad. Seljskohoz. Nauk. 15:24–28. In: An. Breed. Abs. 9:18–19. 1940.
37. *Underbjerg, G. K. L. and H. P. Davis.* Longevity, Fecundity, Motility and pH of Diluted and Undiluted Bovine Semen. Amer. Soc. Anim. Prod. Proc. 33:229–236. 1940.
38. *Underbjerg, Gravers K. L., H. P. Davis and R. E. Spangler.* Effect of Diluters and Storage Upon Fecundity of Bovine Semen. Jour. Anim. Sci. 1:80. 1942.
39. *United States Department of Agriculture.* Report of the Chief of the Bureau of Animal Industry, p. 5, 1942.
40. *Ward, A. H.* What Was Revealed from the Figures. N. Z. Jour. Agric. 61:179–182. 1940.
41. *Werner, G. M., L. E. Casida and I. W. Rupel.* Estrus, Ovulation, and Artificial Insemination in Cattle. Amer. Soc. Anim. Prod. Proc. 31:54–57. 1938.
42. *Willett, E. L., H. K. Fuller and G. W. Salisbury.* Preservation of Bovine Spermatozoa in Yolk Phosphate Diluent and Field Results from Its Use. Cornell Vet. 30:507–513. 1940.
43. *Zupp, B. A.* The Cyclic Secretory Phenomena of Oestrus in the Cow. Vet. Pract. Bul., Iowa State College 8:123–152. 1926.